IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Wynick, David	)	Art Unit: 1647
	)	
Application No. 09/230,463	)	Examiner: Gucker, S.
	)	
Filing Date: January 22, 1999	)	Confirmation No. 4323
	)	
For: "GALANIN"	)	

DECLARATION UNDER 37 C.F.R. § 1.132

1. I am Professor in the Department of Neurosciences at Case Western Reserve University, Ohio, USA.

2. I have been associated with teaching and research in the subject of Neurosciences for almost thirty years and have published approximately 100 peer-reviewed papers and 25 review articles and chapters during this time. Examples of these publications together with details of my education are given in the short version of my *curriculum vitae* which is attached and shown as Exhibit A.

3. My research relates to neurochemical plasticity in adult neurons. In recent years, my laboratory has focused on the molecules and cells involved in altering neuronal gene expression in response to axonal injury. The galanin peptide has been one molecule of long-standing interest and a major focus of my laboratory.

4. I am familiar with the work of David Wynick in the field of galanin and nerve regeneration.

5. I have reviewed the specification of US Patent Application Serial No. 09/230,463 ("the patent application"). I understand the technology described in the patent application. In

particular, I have reviewed claim 18 of the patent application and its meaning is clear to me.

6. I have also reviewed the publication "The effects of pretreatment with tachykinin antagonists and galanin on the development of spinal cord hyperexcitability following sciatic nerve section in the rat" by Luo, L. and Wiesenfield-Hallin, Z. (1995) Neuropeptides **28** 161-166 ("the Luo publication"). I understand the experiments described in that publication and the implications of the data resulting from those experiments.

7. In addition, I have reviewed the Office Action mailed on 10th March 2005, particularly item 5 of the Action in which the Examiner rejected claims 18 and 25-26 under 35 U.S.C. 103(a) as being unpatentable over the Luo publication in view of Zhang *et al.* (J. Neurocytology (1993) **22** 342-381).

8. The Luo publication demonstrates that galanin inhibits spinal cord electrical hyper-excitability for 60 minutes following nerve section (see Figure 3), at which point the animals were sacrificed. Galanin was administered 30 minutes before the nerve injury as a one-off bolus injection of 2.4nM (low dose) directly into the space surrounding the bottom part of the spinal cord (intrathecal (IT) administration into the lumbar enlargement, see page 163, paragraph headed "Effect of galanin" and Figure 3 of the Luo publication). The Luo publication deals exclusively with neuropathic pain and spinal cord excitability, not with peripheral nerve regeneration as claimed in the patent application. The mechanism by which the galanin rapidly alters pain activity is most likely by direct modulation of spinal cord neuronal firing rather than at the level of the dorsal root ganglion (DRG).

9. When damage or injury to sensory or motor nerves (in this case the sciatic nerve) occurs, this triggers a cascade of molecular events within the cell bodies of that nerve, in this case the DRG, which in turn attempts to repair the damage and restore the normal function of the nerve, so-called nerve regeneration. The Protein Kinase C (PKC) and MAP Kinase (MAPK) intra-cellular signalling cascades have both been shown to up-

regulate after nerve injury and are vital for nerve regeneration (Klinz & Heumann (1995) J. Neurochem. **65** 1046-53; Kiryu et al. (1995) Brain Res. Mol. Brain Res. **29** 147-56). At the 1996 priority date of the patent application, no published literature existed to indicate that galanin speeded up nerve regeneration, nor that it activated PKC or MAPK.

10. For regeneration to have occurred in the animals used in the experiments documented in the Luo publication during the 90 minute period before the animals were sacrificed, the galanin would have had to gain access to the cell bodies in the DRG, since this is where the intra-cellular pro-regenerative machinery resides (Terenghi (1994) J. Anatomy (Pt I) 1-14). The only way that galanin, when applied to the space surrounding the bottom part of the spinal cord, could have reached the cell bodies of the sensory neurons in the DRG which is where "...the preliminary beginnings of regeneration..." would have occurred, is by direct uptake of the galanin by the nerve terminals in the dorsal horn of the spinal cord. The cell bodies of the sensory neurons of the DRG lie outside the central nervous system (CNS), whilst the spinal cord is part of the CNS. The cerebrospinal fluid (CSF) that bathes the spinal cord is not in contact with the DRG and thus galanin could not have reached the DRG by passive diffusion.

11. There are well documented and active transport mechanisms in sensory neurons that move proteins from the nerve terminals of the spinal cord or the ends of the peripheral axons of the sciatic nerve to the cell bodies in the DRG, termed retrograde transport. A number of these retrograde transport mechanisms for Nerve Growth Factor (NGF) and Horseradish Peroxidase (HRP) have been extensively studied and characterized. There is good agreement between these published papers that the rate at which these retrograde transport processes move proteins from the rat dorsal horn of the spinal cord to the cell body in the DRG is a maximum rate of 7.5 mm/hour (range 2.5 – 7.5 mm/hr, Yip and Johnson, Jr. (1986) J. Neurocytol. **15** 789-98; Richardson and Riopelle. (1984) J. Neurosci. **4** 1683-9).

12. Michael et al. (J. Neurosci. (1997) **17** 8476-8490) found the nerve root of adult male Wistar rats (200-400g body weight) to be 17 mm in length. Similarly, Baba et al. (Baba et al. (1999) J. Neurosci. **2** 859-867) found the dorsal root to be between 18-20 mm in

length in adult male Sprague-Dawley rats weighing 300-350g. Based on this, assuming the maximum rate of retrograde transport of galanin from the dorsal horn of the spinal cord to the DRG is 7.5 mm/hr and the length of the nerve root between the dorsal horn of the spinal cord and the DRG is at least 17mm in length, then the galanin would only have been transported 11.25 mm in the 90 minutes after galanin was administered before the animals were sacrificed (i.e. about two thirds of the way to the DRG). Galanin could not, therefore, have even begun to affect regeneration in the DRG cell bodies by the time the experiment was terminated.

13. In addition, the concentration of galanin would have immediately and rapidly begun to fall straight after the administration of the bolus injection of 2.4nM galanin into the lumbar enlargement of the spinal cord. This would have occurred within seconds, as the galanin would immediately be diluted by the CSF and almost immediately would also have started to be degraded by proteolytic enzymes in the CSF (Bedecs et al. (1995) Neuropeptides **29** 137-43). Therefore, even in the highly unlikely event that a small proportion of the galanin that was administered by bolus-dose to the spinal cord did reach the DRG by retrograde transport, the final dose would be substantially lower (in the sub-nanomolar range) than the originally administered IT (page 163 and Figure 3 of the Luo publication). In contrast, the dose of galanin demonstrated to stimulate nerve outgrowth from sensory neurons by direct application in cell culture to the DRG cell body is 100nM galanin (Mahoney et al. (2003) J. Neurosci. **23** 416-421). In light of this, the effective concentration of galanin that would have reached the DRG would have been at least 100-fold lower than that necessary to stimulate regeneration, again making it highly unlikely that galanin could have even begun to affect regeneration in the DRG cell bodies by the time the experiment was terminated.

14. For these reasons, it is my view that one of ordinary skill in the art would have no incentive, on reading the Luo publication, to imagine that galanin-induced nerve regeneration of the severed sciatic nerve would have begun during the time period utilized in the experiments of Luo et al. In addition, in light of the Luo et al. disclosure and on reading the disclosure in Zhang et al. that galanin may be suitable for use as an

analgesic in humans, one of ordinary skill in the art would have no motivation to administer a galanin agonist in a method for the treatment of peripheral nerve damage, wherein the peripheral nerve damage is treated by nerve regeneration, as claimed in claim 18 as amended with the most recently filed Applicant's response.

15. I further declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 6/1/05

Professor Richard E. Zigmond

EXHIBIT A

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
RICHARD E. ZIGMOND	PROFESSOR OF NEUROSCIENCES

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Harvard College, Cambridge MA	BA cum laude	1962-66	Biology
Rockefeller U., NY, grad. stud.with Bruce McEwen	Ph.D.	1966-71	Neuroscience
Rockefeller U., NY, postdoc.with Don Pfaff		1971-72	Neuroendocrinology
U. of Cambridge, UK, postdoc. with Leslie Iversen		1972-75	Neurochemistry
Harvard Medical School/Children's Hospital, (Sabbatical with Michael Greenberg)		2000-01	Molecular Biology

Positions and Honors

Appointments: Assist. Prof. (1975-81), Assoc. Prof. (1981-89) of Pharmacology, Harvard Medical School; Instructor in Neurobiology of Behavior at Cold Spring Harbor Lab. (1979-82); Prof. of Neurosci., Case Western Reserve Univ. (CWRU) School of Medicine (1989-present); Instructor in Neurobiology at Marine Biology Lab. (1981-84); Program Director, NIH Postdoctoral Training Program in Devel. Neurol. (1981-89; Harvard Med. Sch.); Instructor on Review and Update in Neurobiology for Neurosurgeons (1984, 86, 88); Chair, Committee on Appointments and Tenure, Department of Neurosciences, CWRU (1991-present); Chair, Gordon Conference on Neural Plasticity (1991); Program Committee, Society for Neuroscience (1991-93); Acting Chair, Department of Neurosciences, 1992-93).

Fellowships and Special Grant Awards: Pop. Council Fellowship in Mammalian Reproduction (1971-72); British-American Heart Found Fellowship (1972-73); Sloan Found. Fellowship in Neurochem. (1972-74); Klingenstein Fellowship in the Neurosciences (1987-1990); Mellon Found. Faculty Award (1976-1977); NIMH Research Scientist Development Award (1977-87); Javits Neuroscience Investigator Award (1987-94); NIMH Research Scientist Award (1987-94).

Grant Review Committees: External review committee for the Lab. of Developmental Neurobiology NICHD (1985, 1990); Study Section for Tobacco-Related Disease Research Program of the Univ. of California (1993, 1994); Ad hoc Reviewer, Neurology C Study Section (Neuro C; 1995, 1996); Member, Neurological Sciences 1 Study Section (NLS1) and Molecular Developmental and Cellular Neurosciences Study Section (MDCN7; 1996-2000); Reviewer of Research at the Burke Medical Research Institute (1998).

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Editorial Boards: *J. Neurosci. Meth.* (1978-1998), *J. Neurosci.* (1985-91), *Ann. Rev. Neurosci.* (1986-90), *TINS* (1986-90), *New Biol.* (1989-91), *Adv. in Neurosci.* (1989-present), *Neuroscience* (1996-present), *J. Mol. Neurosci.* (1997-present), *Autonomic Neuroscience: Basic and Clinical* (formerly *J. Auton. Nerv. Syst.* (1998-), *NeuroSignals* (formerly *Biological Signals and Receptors* (2001-present).

Selected peer-reviewed publications since 1998.

1. Rao MS, Sun Y, Escary JL, Perreau J, Patterson PH, Zigmond RE, Brulet P, Landis SC. Leukemia inhibitory factor mediates an injury response but not a developmental transmitter switch in sympathetic neurons. Neuron 11:1175-1185, 1993.
2. Sun Y, Rao MS, Zigmond RE, Landis SC. Regulation of vasoactive intestinal peptide expression in sympathetic neurons in culture and after axotomy: The role of cholinergic differentiation factor/leukemia inhibitory factor. J Neurobiol 25:415-430, 1994.
3. RC, Shadiack AM, Bennett TA, Sedwick CE, Zigmond RE. Changes in the macrophage population of the rat superior cervical ganglion after postganglionic nerve injury. J Neurobiol 27:141-153, 1994.
4. Sun Y, Landis S, Zigmond R. Signals triggering the induction of leukemia inhibitory factor in sympathetic superior cervical ganglia and their nerve trunks after axonal injury. Mol Cell Neurosci 7:152-163, 1996.
5. Hyatt-Sachs H, Bachoo M, Schreiber R, Vaccariello SA, Zigmond RE. Chemical sympathectomy and postganglionic nerve transection similarly increase galanin and VIP mRNA but not the peptides themselves. J Neurobiol 30:543-555, 1996.
6. Sun Y, Zigmond RE. Leukemia inhibitory factor induced in the sciatic nerve after axotomy is involved in the induction of galanin in sensory neurons. Europ J Neurosci 8:2213-2220, 1996.
7. Zigmond, RE. Retrograde and paracrine influences on neuropeptide expression in sympathetic neurons after axonal injury. In: Cytokines and the CNS: Development, Defenses and Disease (RM Ransohoff, EN Benveniste, Eds) CRC Press, Boca Raton, pp. 169-186, 1996.
8. Zigmond RE, Hyatt-Sachs H, Mohney RP, Schreiber RC, Shadiack AM, Sun Y, Vaccariello SA. Changes in neuropeptide phenotype after axotomy of adult peripheral neurons and the role of leukemia inhibitory factor. Perspec Dev Neurobiol 4:75-90, 1996.
9. Zhou Y, Deneris E, Zigmond RE. Differential regulation of levels of nicotinic receptor subunit transcripts in adult sympathetic neurons after axotomy. J Neurobiol 34:164-178, 1998.
10. Shadiack AM, Zigmond RE. Galanin induced in sympathetic neurons after axotomy is anterogradely transported toward regenerating nerve endings. Neuropeptides 32:257-264, 1998.
11. Shadiack AM, Vaccariello SA, Sun Y, Zigmond RE. Nerve growth factor inhibits sympathetic neuron's response to an injury cytokine. Proc Natl Acad Sci USA 95:7727-7730, 1998.
12. Mohney RP, Zigmond RE. Vasoactive intestinal peptide enhances its own expression in sympathetic neurons after injury. J Neurosci 18:5285-5293, 1998.
13. Zigmond RE, Mohney RP, Schreiber RC, Shadiack, AM, Sun Y, Vaccarriello SA, Zhou Y. Plasticity in gene expression in adult sympathetic neurons after axonal damage. Adv in Pharmacol 42:899-903, 1998.
14. Nagamoto-Combs K, Vaccariello S, Zigmond RE. The levels of LIF mRNA in a Schwann cell line are regulated by multiple second messenger pathways. J Neurochem 72:1871-1881, 1999.
15. Mohney RP, Zigmond RE. Galanin expression is decreased by cAMP-elevating agents in cultured sympathetic ganglia. NeuroReport 10:1221-1224, 1999.

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16. Rittenhouse A R., Zigmond RE. The role of N- and L-type calcium channels in the depolarization-induced activation of tyrosine hydroxylase and release of norepinephrine by sympathetic cell bodies and nerve terminals. J Neurobiol 40:137-148, 1999.
17. Boeshore KL, Luckey CN, Zigmond RE, Large TH. TrkB isoforms with distinct neurotrophin specificities are expressed in predominantly non-overlapping populations of avian dorsal root ganglion neurons. J Neurosci 19:4739-4747, 1999.
18. Ip NY, Zigmond RE. Synergistic effects of muscarinic agonists and secretin or vasoactive intestinal peptides on the regulation of tyrosine hydroxylase activity in sympathetic neurons. J Neurobiol 42:14-21, 2000.
19. Bibevski S, Zhou Y, McIntosh J, Zigmond R, Dunlap M. Functional nicotinic acetylchoine receptors that mediate ganglionic transmission in cardiac parasympathetic neurons. J. Neurosci 20:5076-5082, 2000.
20. Zigmond RE. Neuropeptide action in sympathetic ganglia: Evidence for distinct functions in intact and axotomized ganglia. Ann N Y Acad Sci.921:103-108, 2000.
21. Shadiack A, Sun Y, Zigmond R. Nerve growth factor antiserum induces axotomy-like changes in neuropeptide expression in intact sympathetic and sensory neurons. J. Neurosci. 21:363-371, 2001.
22. Zhou Y, Deneris E, Zigmond R. Nicotinic acetylcholine receptor subunit proteins alpha7 and beta4 decrease in the superior cervical ganglion after axotomy. J Neurobiol. 46: 178-192, 2001.
23. Schreiber RC, Krivacic K, Kirby, B, Vaccariello SA, Tani M, Ransohoff RM, and Zigmond RE. Monocyte chemoattractant peptide- 1(MCP-1) is rapidly expressed by sympathetic ganglion neurons following axonal injury. NeuroReport 12:601-606, 2001.
24. Zigmond RE. Can galanin also be considered as growth-associated protein 3.2? TINS 24:494-496, 2001.
25. Takasu, AM. Dalva MB, Zigmond R, Greenberg, ME. Science. EphB receptors modulate NMDA receptor-dependent calcium influx and gene expression. Science 295: 491-495, 2002.
26. Schreiber RC, Boeshore K, Vaccariello SA, Shadiack, Zigmond RE. A comparison of the changes in the non-neuronal cell populations of the superior cervical ganglia following decentralization and axotomy. J. Neurobiol. 53: 68-79, 2002.
27. Zigmond R.E. Plasticity in the autonomic nervous system: Responses of adult sympathetic neurons to injury. In: Handbook of Autonomic Nervous System in Health and Disease (J Licinio and L Bolis, Eds) Marcel Dekker, New York, pp. 167-184, 2002.

Research Support

Sponsor: National Institutes of Health **Award Number:** NS12651
Dates: 7/1/2003-6/30/2004 **Amounts:** Current Direct Costs: 0
Title: Experience and the Neurochemistry of the Synapse
Percent Effort: 35%
Brief description of the project: To identity the transmitter responsible for the non-cholinergic activation of TH in the SCG after preganglionic nerve stimulation, determine whether such nerve stimulation alters neuropeptide expression, determine whether PACAP and VIP are involved in feedback mechanisms regulating their own expression, examine the signals triggering the changes in nAChR receptor subunit mRNA expression in axotomized SCG neurons, determine if changes occur at the receptor level, and ask whether a phenomenon comparable to "disuse supersensitivity" is seen in these receptors as a result of changes in afferent nerve stimulation.

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Sponsor: National Institutes of Health **Award Numbers:** NS17512
Dates: 5/15/03-4/30/07 **Amounts:** Current Direct Costs: $237,500
Title: Recovery of Function after Neural Damage
Percent Effort: 35%

Brief description of the project: To determine the cellular and molecular changes that occur in peripheral neurons in the context of regeneration. We have been notified by the NINDS that this application will be refunded.